12-1962

The Availability of Phosphorus in Two Defluorinated Phosphates to Sheep as Determined by a Radioisotope Technique

Carlen Pippin
University of Tennessee - Knoxville

To the Graduate Council:

I am submitting herewith a thesis written by Carlen Pippin entitled "The Availability of Phosphorus in Two Defluorinated Phosphates to Sheep as Determined by a Radioisotope Technique." I have examined the final electronic copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Animal Science.

O. Glen Hall, Major Professor

We have read this thesis and recommend its acceptance:

M. C. Bell, Charles S. Hobbs, H. J. Smith

Accepted for the Council:
Carolyn R. Hodges

Vice Provost and Dean of the Graduate School

(Original signatures are on file with official student records.)

August 29, 1962

To the Graduate Council:

I am submitting herewith a thesis written by Carlen Pippin entitled "The Availability of Phosphorus in Two Defluorinated Phosphates to Sheep as Determined by a Radioisotope Technique." I recommend that it be accepted for nine quarter hours of credit in partial fulfillment of the requirements for the degree of Master of Science, with a major in Animal Husbandry.

O Glen Hall
Major Professor

We have read this thesis and
recommend its acceptance:

Charles S. Hobbs

H. J. Smith

M. C. Bell

Accepted for the Council:

Hilton A. Smith
Dean of the Graduate School

THE AVAILABILITY OF PHOSPHORUS IN TWO DEFLUORINATED PHOSPHATES TO SHEEP AS DETERMINED BY A RADIOISOTOPE TECHNIQUE

A Thesis

Presented to

the Graduate Council of

The University of Tennessee

In Partial Fulfillment

of the Requirements for the Degree

Master of Science

---

by

Carlen Pippin

December 1962

ACKNOWLEDGMENT

The author wishes to express his sincere appreciation to his major professor, Dr. O. G. Hall, Associate Professor of Animal Husbandry, for his assistance, encouragement, helpful comments, and guidance in carrying out the work involved in this study and in the preparation of this thesis. Without his assistance and encouragement the completion of this study would not have been possible.

He is indebted to Dr. M. C. Bell, Associate Professor of Animal Husbandry and Associate Scientist of the University of Tennessee Agricultural Research Laboratory at Oak Ridge, for making arrangements at Oak Ridge which were necessary for the completion of this study, for his assistance prior to and during the conduction of the metabolism trial and for helpful suggestions in the preparation of this thesis.

Gratitude is expressed to Dr. C. S. Hobbs, Head of the Animal Husbandry-Veterinary Science Department, for his assistance throughout the past year, and to Dr. H. J. Smith, Professor of Animal Husbandry, for his helpful comments in the preparation of this thesis.

The author would like to express his personal appreciation to Dr. N. S. Hall, Director of the University of Tennessee Research Laboratory at Oak Ridge, for the use of the laboratory facilities, and to the Smith Douglas Company, Inc. for supplying part of the funds needed to conduct this research.

C. P.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER I

INTRODUCTION

Phosphorus deficiency has limited livestock production since the beginning of recorded history. This is understandable in view of the essentiality of phosphorus to various life processes and of the problems of providing adequate amounts of available phosphorus in rations for livestock.

One of the best known functions of phosphorus is its importance in building bones and teeth. Also, it has an equally important function in every living cell of the body. It is essential for the utilization and metabolism of the other nutrients such as proteins, fats, and carbohydrates. Muscles cannot grow and divide, and cells cannot move except under the influence of phosphorus. This element is, therefore, very closely associated with the basic nature of living matter.

In the early days of nutrition research, investigators worked long and hard in proving that animals could utilize the inorganic form of many elements, such as phosphorus, as well as their organically bound forms. Later it was rather widely assumed that all inorganic chemical forms of an element such as phosphorus were of equal feeding value. More recent studies have shown that this is not the case, and that a given weight of an element from one source may be less effective than the same amount from another source.

Increased demands for feed phosphorus and shortages of bone meal

have greatly increased the need for other sources of phosphorus, and several different sources are now available.

Among the most common of these sources are defluorinated domestic rock phosphates, dicalcium phosphate, Curacao Island phosphate, and a limited amount of bone meal and phosphoric acid.

The biological availability of phosphorus in these supplements to ruminants has not been widely studied. This is due to the expense and length of time required for conventional metabolism studies. During the past few years an in vitro technique, involving washed rumen microorganisms, has been proposed as a quick and less expensive method of assaying phosphorus supplements. The validity with which results from this technique can be applied directly to the animal has not been determined.

With the advent of the radioisotope dilution technique, the true digestibility of the phosphorus in a supplement can now be determined. The specific objective of the experiments reported in this thesis was to determine the true digestibility of the phosphorus in two defluorinated phosphate supplements using the isotope dilution technique. The apparent availability of the phosphorus in both supplements was determined with washed rumen microorganisms prior to the initiation of the radioisotope study. Thus it was possible to compare the results from both techniques.

CHAPTER II

REVIEW OF LITERATURE

General Methods of Determining Phosphorus Availability

This literature review is primarily concerned with the techniques, procedures, and results of experiments designed to determine the availability of phosphorus in supplements to ruminants and rumen microorganisms. No attempt is made to review the vast literature dealing with the broader aspects of phosphorus metabolism. These aspects are discussed in great detail in a number of extensive reviews (Greenberg, 1939; Glass, 1952; Duckworth and Hill, 1953; and others).

To a large extent, the quality of a phosphate feed material is dictated by the availability of the phosphorus in the material to animals. A number of methods with different criteria of measurement have been used for evaluating phosphates by nutritionists.

Theiler (1928) made one of the earliest evaluations of phosphorus supplements. He observed that bone meal, sodium phosphate, calcium phosphate, phosphoric acid, and wheat bran were effective in alleviating the osteophagia of phosphorus-deficient cattle; ground rock phosphate did not cure the deficiency. Toit et al. (1930) also reported that calcium phosphate was a very effective agent for the cure of osteophagia. Reed and Huffman (1930) reported that feeding raw rock phosphate to cattle produced abnormal teeth, exostosis, and alkylosis. These abnormalities were attributed to high levels of fluorine in the rock phosphate.

Ellis et al. (1945) used bone formation in the rat to determine the availability of calcium and phosphorus in commercial and experimental defluorinated phosphates. Defluorinated rock phosphate compared favorably with bone meal and calcium phosphate in these studies. A rating of good to fair in availability was given to phosphate slag.

Bone formation in the rat was used by Barrentine et al. (1944) to compare three sources of defluorinated phosphates. The phosphorus in one of the defluorinated phosphates was significantly less available to the rat than was the phosphorus in calcium phosphate or bone meal. This supplement inhibited the growth of rats when used at a high level. Another one of the defluorinated phosphates produced bone formation equally as well as calcium phosphate.

Ellis et al. (1945) compared the availability of phosphorus in defluorinated phosphates using bone ash and serum phosphorus as the criteria of measurement. Alpha tricalcium phosphate and beta tricalcium phosphate were found to be excellent sources of phosphorus for the rat and superior to meta and pyro ferric and aluminum phosphates. Also results were given of various feeding tests with commercial phosphates. There was much variability in the samples tested. It was shown that the form in which the calcium phosphate exists has an important bearing on its availability. Calcium metaphosphate of the beta form and beta pyrophosphate were relatively unavailable. The alpha and beta ortho forms of tricalcium phosphates were highly available to the rat.

Although palatability is not the only important consideration in

evaluating phosphorus supplements, it does play a significant role since many supplements are fed free choice. Stoddard and Micklesen (1961) used different sources of phosphorus in conducting several free choice feeding trials with dairy cows. Bone meal was the least palatable, with dicalcium and defluorinated phosphates the more palatable. Dew et al. (1954) proved that the palatability of steam bone meal for cows could be improved by mixing it with salt.

Gullickson et al. (1946) compared defluorinated phosphates and steam bone meal in a phosphorus-deficient dairy ration. Calves were fed similar phosphorus-deficient basal rations during the 17-month period of the experiment. There was no significant difference in availability of the phosphorus from the two sources as measured by feed efficiency and growth rate; no difference in the palatability of the two products was noted.

Wise et al. (1961) used 40 Holstein male calves in two experiments to determine the phosphorus requirements of young calves. Dicalcium phosphate was added to the basal ration to supply total phosphorus levels on an air-dry basis of 0.09, 0.12, 0.18, and 0.30 per cent in one experiment. In a second experiment the dietary phosphorus levels were 0.14, 0.22, 0.30, and 0.38 per cent. Twelve different response criteria were utilized and among them were feed intake and efficiency, body weight gains, serum inorganic phosphorus and alkaline phosphatase activity, bone ash, and bone growth as measured from femur and rib antoradiographs. These workers concluded that the minimum phosphorus requirements of calves weighing from 200 to 275 pounds at 12-18 weeks of

age approximates 0.22 per cent of the air dry ration. This means that feeding standards recommending as high as 0.40 per cent phosphorus for calves of this size may be safely scaled downward. They recommended that 0.30 per cent phosphorus be included in the ration of calves of the size and weight used in these studies. This adds a factor of safety to the minimum requirements.

Hodgson et al. (1948) compared steamed bone meal and defluorinated phosphates as phosphorus supplements for fattening steers. Both proved to be satisfactory supplements when fed in mixed feeds.

Burroughs (1956) reported that cattle gained more rapidly when fed dicalcium phosphate than when fed soft phosphates.

Studies with growing beef cattle by Long et al. (1956), failed to show any significant difference in the availability of phosphorus in steamed bone meal dicalcium phosphate, defluorinated rock phosphate, and Curacao Island phosphate.

Ammerman et al. (1957), using yearling steers, reported no significant difference in the availability of the phosphorus in soft phosphate and that in bone meal, defluorinated rock phosphate, or an imported rock phosphate, using phosphorus balance and blood phosphorus levels as criteria of availability. In contrast, Long et al. (1956) found that the availability of phosphorus from dicalcium phosphate was greater than that from soft phosphate, as measured by bone ash, plasma inorganic phosphorus levels, and weight gained.

Tillman et al. (1956) found that when readily available phosphorus

was fed to phosphorus-deficient cattle and sheep, a rise in the plasma inorganic phosphorus followed. This rise in serum phosphorus appeared to reflect the availability of the phosphorus from the various materials tested as measured by the weight gains of the animals.

Ammerman et al. (1955) used sheep to study the utilization of phosphorus from four inorganic phosphorus supplements. Phosphorus supplements studied included dicalcium phosphate, Curacao Island phosphate, soft phosphate with colloidal clay, and defluorinated rock phosphate. These workers found no difference in the utilization of any of the supplements tested based on blood serum phosphate levels.

Ammerman et al. (1957), working with sheep, found that the phosphorus of gamma calcium pyrophosphate was almost totally unavailable while that supplied by vitreous calcium metaphosphate appeared to be about 50 per cent as available as that of monocalcium phosphate. They also found that gamma calcium pyrophosphate was effective in increasing the plasma inorganic phosphorus levels of sheep and suggested that the phosphorus of this compound was absorbed and subsequently excreted into the gastrointestinal tract; however, they did not study this.

Jordan et al. (1956) were able to obtain quite striking differences in serum phosphorus levels in swine. These differences were brought about by the feeding of different phosphorus supplements. Also a diet very deficient in phosphorus was used. When swine received a very available source of phosphorus such as dicalcium phosphate or monocalcium phosphate, the serum phosphorus increased over the 56-day

experimental period; however, when the colloidal clay was fed, the pig's serum phosphorus level dropped.

Methods generally accepted for assaying inorganic phosphate as a source of phosphorus for chicks have usually taken at least a four week supplemental feeding period (Gillis et al., 1948, 1954; Grau and Zweigart, 1953; Miller and Joukovsky, 1953; Creech et al., 1956; Gardiner et al., 1959). Ammerman et al. (1960) reported that a satisfactory assay with chicks was obtained with a ten-day feeding period which included four days of phosphorus depletion followed by six days of feeding phosphorus-supplemented diets. However, this assay was not as sensitive as the four week assay as a measure of phosphorus availability.

Miller and Joukovsky (1953) compared defluorinated phosphate and bone meal as phosphorus supplements for chicks. Both chick weight and bone ash determinations showed that defluorinated phosphate was equal to dicalcium phosphate and bone meal in availability of the phosphorus.

Gillis et al. (1951), experimenting with chicks, showed considerable variation in the availability of phosphorus from different sources. Bone meal, dicalcium phosphates, defluorinated phosphates, and Curacao Island phosphate showed some variation in the availability of phosphorus, but generally were very satisfactory sources of phosphorus. Colloidal phosphate was an extremely poor source of phosphorus for the chick.

Wilcox et al. (1954) reported that monobasic calcium phosphate and two defluorinated phosphates were excellent sources of phosphorus

for turkey poults based on bone ash determinations.

Reynolds et al. (1944) proposed that in the absence of actual feeding experiments the solubility of different phosphorus supplements in 0.4 per cent hydrochloric acid might be used as a test of phosphorus availability. Gillis, et al. (1948) compared the solubility of phosphates in 0.4 per cent hydrochloric acid and their availability to chickens. They concluded that solubility in 0.4 per cent hydrochloric acid has a limited value in determining availability of phosphorus in a supplement for animals.

Artificial Rumen Technique

Burroughs et al. (1951) found that rumen microorganisms require phosphorus for efficient cellulose digestion. Hall (1955), using the artificial rumen technique, found that rumen microorganisms required between 50 and 100 ppm of phosphorus for maximum cellulose digestion. Therefore, it seemed that an artificial rumen technique could be used for measuring phosphorus availability to ruminants. A close agreement of such artificial rumen studies and actual feeding experiments would reduce the expense and time that are involved in the actual feeding experiments.

Anderson et al. (1956) described a technique for evaluating phosphorus availability in supplemental phosphorus sources to rumen microorganisms using an artificial rumen technique. This assay is based on the fact that phosphorus-depleted rumen microorganisms will rapidly digest cellulose only when supplied with an adequate amount of phosphorus in an available form. Dicalcium phosphate, acidulated

phosphate, bone meal, Curacao Island phosphate, colloidal clay phosphate, and a standard sodium potassium phosphate mixture were compared as sources of phosphorus for rumen microorganisms. Phosphorus in dicalcium phosphate seemed to be as available as the phosphorus in the standard sodium-potassium phosphate mixture. The bone meal and acidulated phosphorus products were intermediate in phosphorus availability. Curacao Island phosphate and the colloidal clay phosphate were less available sources of phosphorus. These workers proposed the artificial rumen technique used in the above experiments as a method for determining the phosphorus availability of phosphorus supplements for ruminants.

Phosphorus in the phytate form is poorly utilized by non-ruminant animals (Gillis et al., 1953; Chapman et al., 1955; Gillis et al., 1957), but appears to be utilized by ruminant animals (Jordan et al., 1906; Mathur, 1953). Later studies indicated that rapid hydrolysis took place in the rumen (Reid et al., 1947) and that washed suspensions of rumen microorganisms (Raun et al., 1956) were able to produce phytase, suggesting that phytin phosphorus had been completely hydrolyzed by enzymes produced by the rumen microorganisms.

Baxter (1957) and Hall et al. (1961) used an artificial rumen technique to study the effect of ortho, meta, pyro, and phytin phosphates upon cellulose digestion by rumen microorganisms. The amount of cellulose digested in a 20-hour fermentation period was used as the criterion to evaluate microbial activity which was in turn used as an indication of the availability of the phosphorus in sodium orthophosphate,

sodium pyrophosphate, sodium hexa-metaphosphate, and calcium phytate. All sources were tested simultaneously at 0, 20, 40, and 60 micrograms per milliliter of basal medium. The phytate was less available than the other sources at the 20 and 40 micrograms per milliliter levels. At the 20 micrograms per milliliter level, the pyro form was significantly more available than the meta form. The phosphorus from pyrophosphate was slightly more available at the 40 micrograms per milliliter level than a similar level of metaphosphate. Differences in cellulose digestion were not significant when phosphorus from all sources was added at a level of 60 micrograms per milliliter.

Gaddy (1960) and Hall et al. (1961) used washed suspensions of rumen microorganisms, with cellulose digestion in vitro as the criterion of evaluation, to determine the availability of the phosphorus in various phosphorus supplements. The phosphorus in two sources of dicalcium phosphate tried were highly available to the rumen microbes. The phosphorus in Curacao Island phosphate and a defluorinated phosphate was apparently unavailable. These workers found that feeding the defluorinated phosphate to a fistulated steer for 30 days before conducting availability studies failed to alter the availability of the phosphorus in the supplement to rumen microbes.

Radioisotope Technique

Recent advances in radioisotope procedures permit the separation of fecal endogenous phosphorus from that portion that was not absorbed from the gastrointestinal tract. Kleiber et al. (1951), Lofgreen and Kleiber (1953), Comar et al. (1953), and Visek et al.

(1953) used the isotope dilution technique to determine endogenous fecal phosphorus. Knowledge of the true digestibility of phosphorus from different sources in the diet of the animal has been greatly increased by the use of such procedures. Nutritionists have also learned more about the fecal excretory mechanism under various dietary and physiological conditions.

Lofgreen and Kleiber (1953), using the isotope dilution technique, showed the true digestibility of phosphorus in lucerne to be as high as 90 per cent, whereas conventional procedures suggested an apparent digestibility of only 20 per cent.

LeGrande and Tillman (1961) used four wethers which were five to six months of age to determine the availability of phosphorus in wheat bran. Fecal endogenous phosphorus was determined by the isotope dilution procedure, using a single injection of phosphorus-32 at the onset of the collection period. Conventional procedures were used to determine apparent digestibility. The apparent and true digestibilities of phosphorus were 14.75 and 25.49 per cent, respectively. Values for urinary phosphorus were abnormally high in all animals. In this study the specific activity of the urinary phosphorus was essentially the same as that of plasma indicating that the phosphorus of the urine had been available for body utilization.

Tillman and Brethour (1958a) used 12 wethers approximately 18 months old and weighing 90 pounds each to compare two phosphorus sources--calcium phytate and monocalcium phosphate. Both phosphorus supplements supplied 70 per cent of the phosphorus in rations supplying 2.38 grams

of phosphorus per 100 pounds of liveweight. True digestibilities, net retention, apparent digestibilities, and fecal endogenous excretions were used as criteria of availability. These workers found that both the calcium and phosphorus in calcium phytate were as available as the phosphorus and calcium contained in monocalcium phosphate.

Tillman and Brethour (1958b) in a similar experiment studied the utilization of sodium meta-, ortho-, and pyrophosphate by ruminants. Monosodium phosphate was used as the control. These supplements supplied 66.1 per cent of the phosphorus in a ration supplying 2.0 grams of phosphorus per 100 pounds of liveweight. There was no difference in the availability of phosphorus in acid sodium pyrophosphate and monosodium phosphate. Sheep fed vitreous sodium metaphosphate excreted more endogenous phosphorus than those fed the other supplement. There was no difference in the true digestibility of the phosphorus in the three phosphates. These data indicated that although the phosphorus of vitreous sodium metaphosphate was absorbed, it was inefficiently utilized.

Tillman and Brethour (1958c), in a similar experiment with cattle, also found that the availability of the phosphorus in phosphoric acid was as great as that of dicalcium phosphate.

Smith and Wise (1957) reported a bone growth method using autoradiographs and calcium-45 for determining the availability of phosphorus in a phosphorus supplement.

Lofgreen (1960), using the isotope dilution technique, studied the availability of phosphorus in dicalcium phosphate, bone meal, soft

phosphate, and calcium phytate to sheep. True digestibility was used as the criterion of availability. The true digestibilities of the phosphorus in the supplements were 50, 46, 14, and 33 per cent for dicalcium phosphate, bone meal, soft phosphate, and calcium phytate, respectively. Although significantly less phosphorus was absorbed from the soft phosphate than from the other supplement, the phosphorus retained from the basal ration plus soft phosphate was equal to the others. Surplus phosphorus absorbed from the other rations plus the respective supplement was excreted by way of the metabolic fecal phosphorus. Significantly less phosphorus was absorbed from calcium phytate than from dicalcium phosphate and bone meal.

CHAPTER III

EXPERIMENTAL PROCEDURE

This investigation consisted of a metabolism trial in which the true digestibility by sheep of the phosphorus in two defluorinated phosphate supplement was determined by means of the radioisotope dilution technique. The apparent availability of the phosphorus in the two supplements had been determined by means of the artificial rumen technique. These studies have shown that one of the defluorinated phosphate supplements (supplement A) was a very available source of phosphorus whereas the phosphorus in the second supplement (supplement B) was apparently unavailable to cellulose digesting bacteria *in vitro*. Since the *in vitro* results have a direct and very important relationship to the present study some of the data obtained by Satchidanandam (1961) are included in the subsequent section of this thesis so that a comparison of *in vitro* and *in vivo* results is possible.

The defluorinated phosphate supplements used were originally obtained from the open market by sampling three to five bags. The content of phosphorus was determined. Supplement A contained 20.0 per cent phosphorus, and supplement B contained 20.7 per cent phosphorus.

Metabolism Trial

Twelve wether lambs of similar type and condition were selected

from a flock of 16 and used in this experiment. They ranged in weight from 54 to 69 pounds. Six lambs were randomly assigned to each of the two treatments studied.

The lambs were placed in dual-unit type metabolism stalls as described by Hansard (1951). The urine was conducted by funnels to jars with cheese cloth strainers. The cheese cloth kept any fecal material from entering into the urine jars. The fecal material was collected in paper-lined, removable aluminum boxes, held in position at floor level.

For the first 17 days of the 23 day preliminary period, the lambs were kept in metabolism stalls as described above at the Animal Sciences Building. After 17 days the lambs were transported to the UT-AEC Laboratory at Oak Ridge where they were weighed and placed in similar metabolism stalls. An additional five day preliminary period was employed at this location before the lambs were dosed with phosphorus-32 and the seven day collection period started.

The lambs were fed the low-phosphorus, semi-purified ration shown in Table I throughout the experimental period. This ration contained 0.032 per cent phosphorus. The lambs were fed and watered at 8 A.M. and 5 P.M. daily. Approximately 900 grams of the basal ration were fed per head daily.

In addition to the basal ration, six lambs each received 1.5 grams of phosphorus from defluorinated phosphate supplement A and each of six lambs received 1.5 grams of phosphorus from supplement B per day. The phosphorus supplements used were the same as those tested

TABLE I

BASAL RATION

Constituent	Amount
	Per cent
Cottonseed hulls	35.0
Solka-floc (wood cellulose)	30.0
Cerelose	5.0
Corn starch	16.5
Cottonseed oil	3.0
Molasses	3.0
Urea	4.0
Alfalfa meal	3.0
Trace mineral salt	0.5
Vitamin A[a]	+
Vitamin D[b]	+
Total	100.0

[a]Vitamin A supplement containing 10,000 I.U. per gram was added at a rate of 20 grams/100 pounds.

[b]Vitamin D supplement containing 3,000 I.U. per gram was added at a rate of 7 grams/100 pounds.

with rumen microbes. The supplement was weighed very accurately and sprinkled over the feed at the time of feeding.

Since the lambs only received 0.3 gram of phosphorus per head daily from the basal ration, the supplement supplied 83.3 per cent of the phosphorus being injested. Calcium intake by both groups of lambs was equal.

After the first 13 days of the experimental period the animals were eating all their feed with no marked tendency to scour. Certain animals eating supplement B had shown a slight tendency toward loss of appetite early in the experimental period. However, all animals were in good condition at the end of the preliminary period as indicated by the physical state of the feces and by uniform feed consumption.

The administration of phosphorus-32 was made on the first day of the balance trial period. Three of the six animals (selected at random) in each experimental group were given approximately 750 microcuries of phosphorus-32. The isotope was administered intravenously into the jugular vein. The other six lambs received the same amount of the isotope administered orally by means of gelatin capsule and balling gun. Hansard et al. (1951) have described the dosing techniques used in detail with only slight modifications. An aliquot of the phosphorus-32 dosing solutions was transferred into a 250 milliliter flask by the use of the same hypodermic syringe used in measuring the doses. This solution was diluted and used as a counting standard in all subsequent radioactivity measurements.

Daily collections of fecal material were weighed and transferred to plastic bags. After very thorough mixing, approximately 100 grams were stored in screw-topped jars in a refrigerator for further sub-sampling. The feces were homogenized in a mixer before the sub-sample was taken. The urine was weighed and approximately 100 grams were stored in screw-topped jars in a refrigerator until further sub-sampling.

Approximately 20 milliliters of blood was taken from the jugular vein of all animals at approximately 24 hour intervals for seven days starting 24 hours after the animals were dosed. These samples were centrifuged in heparinised graduated tubes for 20 minutes at a speed of 1500 r.p.m. and then stored in a refrigerator. The blood samples were drawn primarily to provide information on the disappearance of the administered nuclide from the blood. The cell volume (hematocrit) was measured on blood samples for the last three days of the trial period. Also, the blood samples from the last two days of the trial period provided blood equilibrium data for use in calculating the endogenous loss of phosphorus by the isotope dilution technique.

Samples and standards were counted for phosphorus-32 activity as solutions and by using a volume of standard equal to that of the samples, the necessity of correcting for self-absorption was thus eliminated.

One milliliter of plasma was added to 5 milliliters of 5 per cent trichloracetic acid in a small centrifuge tube and thoroughly mixed. The solution was centrifuged and a 2-3 milliliter aliquot

of the supernate was analyzed for inorganic phosphorus by the method of Fiske and Subbarow (1925). Total phosphorus in the plasma was determined on the last three of the seven day trial by the method of Lyke (1959).

Radioassay of phosphorus-32 in the plasma was conducted by counting a 3 to 5 milliliter aliquot of the plasma in a small petri dish.

Duplicate samples of approximately 20 grams of fresh feces were dried at 100° C. and ashed in a furnace at 600° C. until a white ash was obtained. The ash was dissolved in concentrated hydrochloric acid and made to a volume of 20 milliliters. A large amount of insoluble residue settled to the bottom of the tubes after being thoroughly mixed. Duplicate 0.01 milliliter aliquots of the supernate was drawn for analysis for phosphorus by the method of Fiske and Subbarow (1925).

Radioassay of the phosphorus-32 in the feces was performed on 5 milliliters of the ash solution as described for plasma.

Duplicate samples of 20 milliliters of urine were evaporated to dryness and ashed at 600° C. The ash was dissolved in 6N hydrochloric acid and diluted to 25 milliliters. Phosphorus was determined on duplicate 5 milliliter aliquots of the urinary ash solutions.

Radioassay of phosphorus-32 in the urine was made directly on duplicate 10 milliliter aliquots of the fresh urine.

Calculation of Data

The method used to calculate endogenous fecal phosphorus and true digestibility of the phosphorus has been described by Thompson (1957). The method used was as follows: Endogenous fecal phosphorus

(E) in grams was determined from the mean specific activities for the feces (SA_f) and plasma (SA_p) on the last two days of the seven day balance trial:

$$E = \frac{SA_f}{SA_p} \quad x \quad \text{daily fecal excretion}$$

The specific activity was expressed as counts per minute per milligram of phosphorus per milliliter of plasma and per gram of feces.

The true digestibility was calculated as follows:

Percentage true digestibility =

$$100 - \frac{(\text{daily output of element (grams)} - E) \times 100}{\text{daily intake of element (grams)}}$$

Analysis of variance techniques as described by Snedecor (1956) were used to statistically analyze the data obtained in these experiments.

CHAPTER IV

RESULTS AND DISCUSSION

Rumen Microbial Experiment

Results of the *in vitro* study by Satchidanandam (1961) in which the availability of the phosphorus to rumen microorganisms was determined is summarized in Table II. It is apparent from data in this table that phosphorus from source B failed to enhance rumen microbial activity as measured by cellulose digestion. Actually cellulose digestion in tubes with added phosphorus from this source was less than that in tubes with no added phosphorus. However, the differences were not statistically significant.

Whereas phosphorus additions from supplement B failed to improve microbial cellulolytic activity, similar additions from supplement A resulted in marked increases in the amount of cellulose digested by the rumen bacteria. Thus, in control tubes only 45 per cent of the cellulose was digested as compared to 58 per cent in tubes with 20 micrograms of phosphorus per milliliter of nutrient solution. An additional significant increase in cellulose digestion resulted when 40 micrograms of phosphorus per milliliter of nutrient solution were added. Apparently this met the requirements for phosphorus by the bacteria since a higher level (80 micrograms per milliliter) did not result in a further increase in cellulolytic activity.

The results of the above experiment are in complete agreement

TABLE II

EFFECT OF DIFFERENT LEVELS OF PHOSPHORUS FROM TWO DEFLUORINATED PHOSPHATE SUPPLEMENTS UPON CELLULOSE DIGESTION BY RUMEN MICROORGANISMS IN VITRO[a]

Source	Amount of P added	Experiment			Average[b]
		1	2	3	
	Mcg/ml. of medium	Cellulose digested (per cent)			
Supplement A	0	46	46	43	45
	20	62	64	48	58
	40	79	72	59	70
	80	79	77	63	73
Supplement B	0	46	46	43	45
	20	43	41	36	40
	40	41	37	38	39
	80	39	41	37	39

[a]Data from Satchidanandam (1961).

[b]Each value is an average of six observations.

with those previously reported by Gaddy (1960) who tested these same phosphate supplements using the rumen microbial technique. Thus, based on both series of experiments it is apparent that phosphate supplement B was a poor source of phosphorus for rumen bacteria whereas supplement A was an excellent source of phosphorus for cellulolytic rumen bacteria *in vitro*.

Metabolism Trial

As previously indicated, the main purpose of this study was to determine the true digestibility of the phosphorus in two defluorinated phosphate supplements which had been extensively studied using the artificial rumen technique. The attempt was to obtain information which would help to determine to what extent *in vitro* results could be extrapolated to the animal.

The phosphorus content of the feces, urine, and plasma is summarized in Table III. The phosphorus content in feces excreted by lambs fed supplement A was 209 milligrams per cent as compared to 186 milligrams per cent in that excreted by lambs fed supplement B. Phosphorus content of the urine from animals receiving phosphorus A was almost two times as great as that in urine from lambs fed supplement B. However, this was almost entirely due to the much higher concentration of phosphorus in the urine of one lamb on the supplement A treatment. Exclusive of this one lamb, there were no significant differences in urinary phosphorus excretion among the other animals. A kidney infection or some other kidney malfunction was suspected as a possible cause of the abnormal phosphorus excretion

TABLE III

PHOSPHORUS CONTENT OF FECES, URINE AND PLASMA[a]

Treatment	Sheep no.	Wet feces	Urine	Plasma[b]	
				Total P	Inorganic P
Supplement A	2	240	0.878	12.75	4.89
	4	184	6.931	14.50	9.27
	6	202	0.886	13.40	7.50
	5	213	1.534	15.70	4.58
	8	233	0.590	13.80	7.25
	10	184	1.254	15.00	5.70
	Mean	209	2.012	14.19	6.67
Supplement B	1	211	1.053	15.50	6.25
	7	205	0.961	15.25	7.30
	11	160	0.883	15.00	4.70
	3	197	1.372	16.00	8.32
	9	175	1.126	15.40	5.52
	12	165	1.000	15.50	6.60
	Mean	186	1.066	15.44	6.43

[a]Expressed as milligram per cent.

[b]Average of last two collection days.

by the lamb. However, no obvious reasons were in evidence.

Very little difference was obtained in the total plasma phosphorus or inorganic plasma phosphorus between treatments. Thus, the total plasma phosphorus was 14.19 for lambs fed supplement A as compared to 15.44 for lambs fed supplement B. The inorganic plasma phosphorus for animals fed supplement A was 6.67 milligrams per cent as compared to 6.43 milligrams per cent for animals that received supplement B.

Daily fecal excretion and radiochemical data of feces, urine, and plasma are shown in Table IV. The lambs that received supplement A excreted 791 grams of feces daily as compared to 866 grams by lambs fed supplement B. The counts per minute per gram of feces excreted were 929 for lambs fed supplement A and 748 for lambs fed supplement B.

Almost twice as much urine was excreted daily by animals receiving supplement A as compared to animals fed supplement B. Also, the counts per minute per milliliter of urine excreted was two times as great by lambs that received phosphorus supplement A as compared to the lambs that received supplement B. However, as already explained, this latter difference was due to the abnormally high excretion of phosphorus in the urine by one of the animals on the A treatment.

The counts per minute per milliliter of plasma for animals receiving supplement A was 88.9 as compared to 112.6 for sheep receiving supplement B. These data indicate (as will be explained in more detail later) that lambs fed supplement A excreted more phosphorus into the gastrointestinal tract than did animals fed phosphorus supplement B.

Phosphorus-32 levels in the blood plasma in both intravenously

TABLE IV

EXCRETION AND RADIOCHEMICAL DATA[a]

TMT	Animal no.	Method of dosing	Feces		Urine		Counts per min/ml plasma
			Daily excretion (gm.)	Counts/ min/gm.	Daily excretion	Counts/ min/ml.	
	2	Oral	788	1276	1194	2.1	82.5
	4	Oral	833	657	1207	43.0	77.9
Supp.	6	Oral	766	1247	813	3.5	74.1
	5	I.V.	703	948	1140	6.4	108.4
A	8	I.V.	643	796	1583	2.4	93.9
	10	I.V.	1015	647	791	2.7	87.1
	Mean		791	929	1121	10.0	88.9
	1	Oral	662	1121	750	3.6	79.1
	7	Oral	826	855	1126	2.5	105.4
Supp.	11	Oral	1025	732	567	3.9	94.7
	3	I.V.	874	659	360	6.5	140.9
B	9	I.V.	793	477	729	7.6	152.3
	12	I.V.	1014	645	559	4.2	103.3
	Mean		866	748	682	4.9	112.6

[a]Average of the seven day collections.

and orally dosed animals at various times following the administration of the radionuclide are shown in Figure 1. These data were calculated from the blood phosphorus-32 concentrations and the total blood volume as described by Hansard et al. (1953).

The disappearance of intravenously administered phosphorus-32 from the blood followed a characteristic curve, and was similar to that for lambs used in experiments by Thompson (1957) and Schroder (1957). Approximately four days after the administration of the dose, the disappearance curve tended to flatten out. This indicated that equilibrium had occurred which would justify the use of data for feces excreted during the last two days of the trial for calculating endogenous fecal phosphorus excretion. Throughout the entire trial period, intravenously dosed lambs that were fed supplement B had an average of approximately 25 per cent more phosphorus-32 in their plasma than lambs that were fed supplement A.

The disappearance of phosphorus-32 from the blood of orally dosed animals is also shown in Figure 1. Similar data have been presented by Thompson (1957). In general, this curve shows more variability between treatments than that for the intravenously dosed animals. There was no significant difference in phosphorus-32 disappearance from the blood of orally dosed animals when the two treatments were compared.

The cumulative levels of fecal phosphorus-32 throughout the trial period for both orally and intravenously dosed animals are shown in Figure 2. Approximately 40 per cent of the orally administered

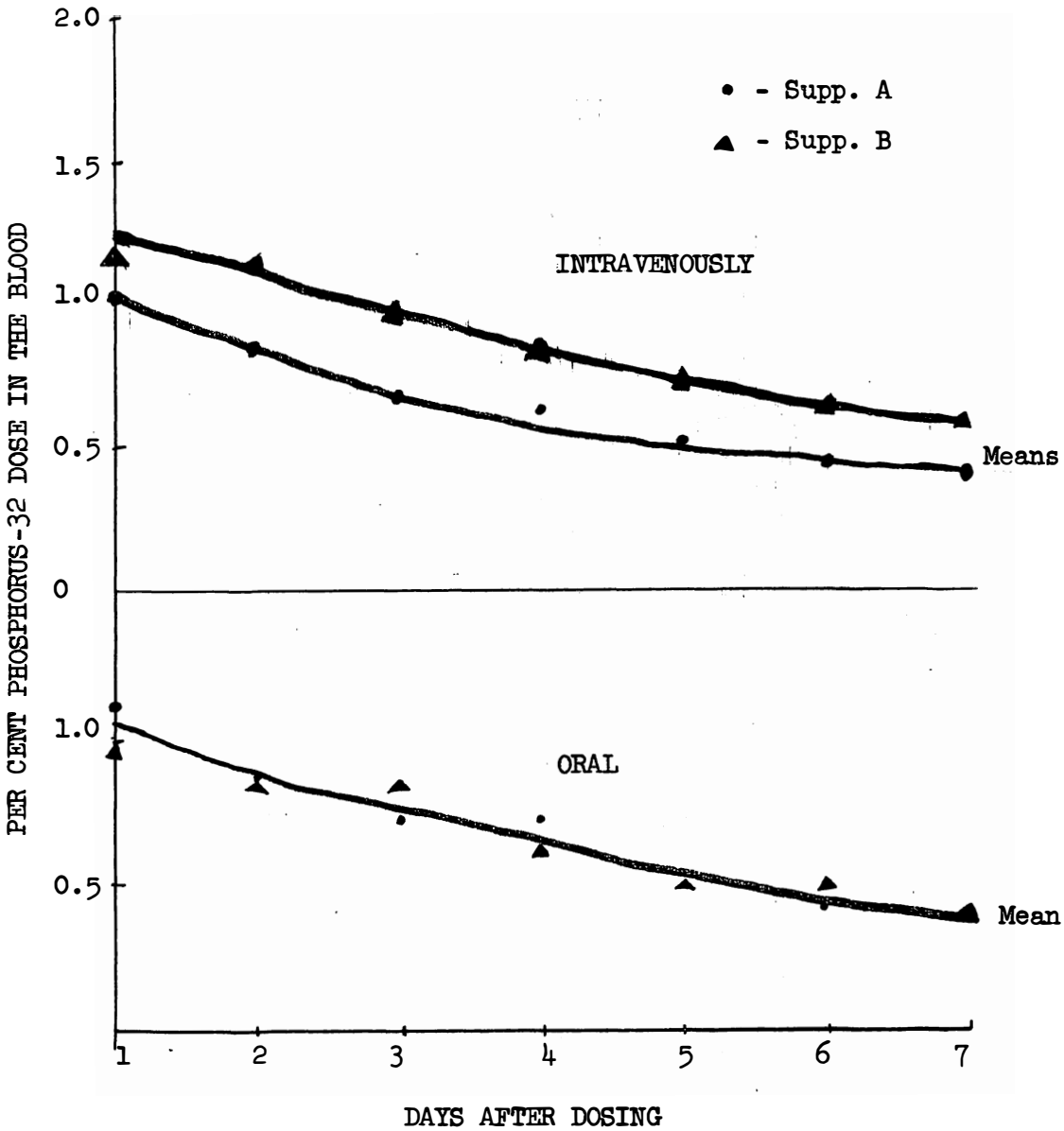

Figure 1. Phosphorus-32 in the blood plasma, following oral and intravenous administration.

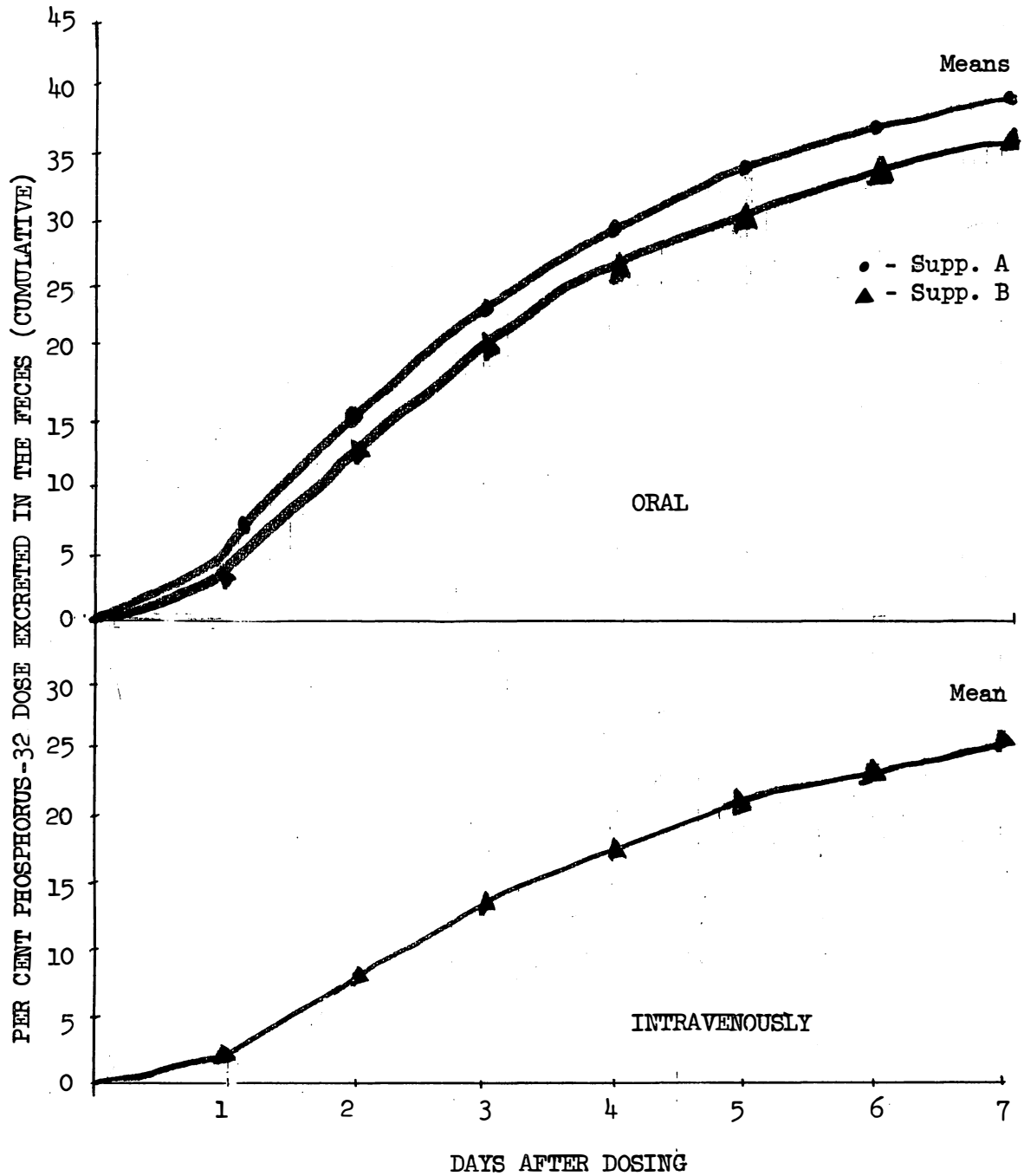

Figure 2. Cumulative fecal excretion of orally and intravenously administered phosphorus-32.

phosphorus-32 appeared in the feces of the animals receiving supplement A and 36 per cent for animals receiving supplement B. Data for the first four days on this curve primarily represent the non-absorbed phosphorus-32; after this it mainly reflects that absorbed and then re-excreted into the gastrointestinal tract as endogenous excretion. These data would suggest a slightly greater excretion of phosphorus by those animals receiving supplement A than for those fed supplement B. There was no difference in fecal excretion of phosphorus-32 by the animals that had been intravenously dosed, irrespective of treatment.

As shown in Table V the phosphate supplements fed to the lambs supplied 83.3 per cent of the total phosphorus in the ration or 1.5 grams of phosphorus per head daily irrespective of treatment. All lambs received 0.3 gram of phosphorus per head daily from the feed. All animals maintained their body weight throughout the metabolism trial. Therefore, the ration supplied enough nutrients to maintain the lambs during this period.

All of the lambs were in positive phosphorus balance that were fed supplement B while two of the animals that were fed supplement A were in a very slight negative phosphorus balance. The average net retention was 0.16 gram for lambs fed supplement A and supplement B. These results on total phosphorus balance indicate about equal availability of the phosphorus in the two supplements. The above statement is also supported by the apparent digestibility of the phosphorus in the two supplements. Thus, while these values were very low, there was no significant difference in them. The actual values were 10.00 per

TABLE V

TOTAL PHOSPHORUS BALANCE DATA[a]

Treatment	Animal no.	Body weight lbs.	Daily intake		Fecal excretion	Urinary excretion	Total excretion	Balance
			Supp.	Feed				
	2	65	1.5	0.3	1.81	0.008	1.82	-0.02
Supplement	4	67	1.5	0.3	1.52	0.090	1.61	0.19
	6	56	1.5	0.3	1.54	0.018	1.56	0.24
A	5	68	1.5	0.3	1.50	0.008	1.51	0.29
	8	62	1.5	0.3	1.50	0.010	1.51	0.29
	10	60	1.5	0.3	1.85	0.008	1.86	-0.06
	Mean	63	1.5	0.3	1.62	0.023	1.64	0.16
	1	59	1.5	0.3	1.67	0.007	1.68	0.12
Supplement	7	63	1.5	0.3	1.68	0.006	1.69	0.11
	11	54	1.5	0.3	1.64	0.008	1.65	0.15
B	3	56	1.5	0.3	1.72	0.007	1.73	0.07
	9	59	1.5	1.3	0.010	0.010	1.39	0.41
	12	66	1.5	0.3	1.66	0.007	1.67	0.13
	Mean	60	1.5	0.3	1.62	0.007	1.63	0.16

[a]Balance data expressed in grams per day.

cent and 9.44 per cent, respectively, for phosphate supplement A and B (see Table VI).

Whereas the above results indicate that both phosphorus supplements were equal in value to sheep, data on the per cent endogenous phosphorus and true digestibility of the phosphorus showed a marked difference in the availability of the phosphorus in the supplements to sheep. As calculated by the radioisotope dilution technique, the fecal endogenous phosphorus was 59.50 per cent for lambs fed supplement A and 38.90 per cent for lambs fed supplement B (see Table VI). Expressed as milligrams of phosphorus per kilogram of bodyweight per day, the values were 34.00 and 22.54 for lambs fed supplement A and B, respectively (see Table VII). The differences between supplements were statistically highly significant.

When the above values were used to calculate the true digestibility of the phosphorus in the supplements, they were 64.0 per cent for supplement A and only 44.6 per cent for supplement B. The difference in these values was also statistically highly significant.

A discrepancy was noted between the results for true digestibility of phosphorus calculated by the comparative balance technique described by Hansard (1952) and Comar et al. (1953) and by the isotope dilution technique of Hansard (1956). The comparative balance technique gave a mean true digestibility of the phosphorus in supplement A of 82.6 per cent and 86.7 per cent for supplement B. According to this technique there was no difference in the true digestibility of the phosphorus in the two supplements. The validity of the comparative

TABLE VI

PHOSPHORUS ABSORPTION AND EXCRETION BY SHEEP
FED TWO DEFLUORINATED PHOSPHATES

	Defluorinated phosphate	
	A	B
Number of animals	6	6
Mean weight (6/14)	62	60
Mean weight (6/26)	63	60
Chemical balance data (gm. daily):		
Intake		
Basal diet	0.300	0.300
Supplement	1.500	1.500
Excretion		
Feces	1.620	1.630
Urine	.023 (.009)[a]	0.007
Net retention	0.157	0.163
Per cent P-32 in feces		
Intravenously administered	26.71	25.62
Orally administered	39.47	36.53
Fecal endogenous phosphorus, per cent	59.50	38.90**
Blood plasma phosphorus (mg./gm.)	0.067	0.064
Apparent digestibility, per cent	10.00	9.44
True digestibility, per cent	64.00	44.60**

[a]Average value for five lambs on this treatment.

**$P < .01$.

TABLE VII

PHOSPHORUS-32 BALANCE DATA

Treatment	Animal no.	Animal weight (lb.)	Method of dosing	Per cent dose in feces[a]	Specific activity[b]		Per cent fecal endogenous	Milligrams of fecal endogenous[c]
					Feces	Plasma		
	2	65	Oral	46.8				
	4	67	Oral	26.1				
Supplement	6	56	Oral	44.9				
	5	68	I.V.	22.5	222	426	52.1	25.28
A	8	62	I.V.	25.7	220	348	63.2	33.74
	10	60	I.V.	31.9	177	280	63.2	42.99
	Mean	63		33.1			59.5	34.00
	1	59	Oral	39.5				
	7	63	Oral	33.3				
Supplement	11	54	Oral	36.6				
	3	56	I.V.	27.6	178	507	35.1	23.77
B	9	59	I.V.	18.5	210	620	33.9	17.46
	12	66	I.V.	31.7	175	367	47.7	26.39
	Mean	60		31.2			38.9	22.59

[a]Per cent of the administered dose excreted during the seven day balance period.

[b]Expressed as counts per gram per minute per milligram of phosphorus.

[c]Expressed as milligrams per kilogram of body weight per day.

balance technique has been questioned by Thompson (1957) and Tillman and Brethour (1958c). These workers believe that the orally administered radioactive element and the stable form in the feed must reach complete equilibrium before independent absorption of the active element takes place. In view of the many phosphorus-containing organic compounds present in biological materials, it seems likely that some of the dietary phosphorus exists in forms not readily exchangeable with administered phosphorus-32. If this were true, the true digestibility of phosphorus pertains only to the true digestibility of the exchangeable fraction of the dietary phosphorus and not to the dietary phosphorus as a whole. It is generally considered that the isotope dilution technique is more valid in determining true digestibility.

Based on the true digestibility values as determined by the isotope dilution technique, the results of the experiments presented in this thesis indicate that the in vitro rumen microbial technique can yield results which are of significance to the live animal. Thus a source of phosphorus which markedly improved in vitro cellulose digestion by rumen bacteria was found to be significantly more digestible by sheep than was a phosphorus source which failed to enhance in vitro cellulose breakdown by rumen microbes. It is conceivable that a phosphorus source could be highly digestible and still not be a utilizable source of phosphorus for life processes. However, based on other tests not reported in the literature, phosphate supplement A is considered to be a very good source of phosphorus for animals.

The fact that the apparently unavailable source of phosphorus

(to bacteria) was utilized to a slight extent by sheep might be explained by the fact that phosphorus in the animal's digestive tract is exposed to a relatively strong hydrochloric acid solution in the abomasum. This might alter to some extent the availability of the phosphorus in a supplement to the animal's body.

During the course of this study an attempt was made to determine what effects the suspension of phosphate supplement B in several solutions of different pHes would have on the availability of the phosphorus to rumen bacteria. However, this attempt did not yield conclusive results.

CHAPTER V

SUMMARY

Twelve wether lambs were used in a metabolism experiment to determine the true digestibility of the phosphorus in two defluorinated phosphate supplements using the radioisotope dilution technique. The apparent availability of the phosphorus in these same supplements had been previously determined by using washed suspension of rumen microorganisms. Results of the *in vitro* rumen microbial studies showed that one of the defluorinated phosphates (supplement A) was a highly available source of phosphorus for rumen microbes whereas the other defluorinated phosphate (supplement B) was an apparently unavailable source of phosphorus for cellulose-digesting rumen microbes.

The lambs were fed a semi-purified ration containing 0.032 per cent phosphorus for 23 days before the seven day collection period was started. In addition to the basal ration, six lambs were fed 1.5 grams of phosphorus from supplement A per head daily and six lambs received a similar level of phosphorus from supplement B throughout the experimental period. All lambs were dosed with 750 microcuries of phosphorus-32 at the beginning of the collection period. Chemical and radiochemical balance data for stable phosphorus and phosphorus-32, blood data for the stable and radioisotope of phosphorus, net retention and apparent and true digestibility of the phosphorus in the two supplements were calculated.

Results on net retention of phosphorus, plasma levels of inorganic phosphorus and of total phosphorus, and apparent digestibility indicated that both supplements were equally available sources of phosphorus for sheep. However, as calculated by the isotope dilution technique, the true digestibility of the phosphorus in supplement A was 60.0 per cent as compared to only 44.6 per cent for the phosphorus in supplement B. The difference in these values was statistically highly significant. Thus, these data indicate that the in vitro rumen microbial technique for determining the apparent availability of phosphorus in supplements can yield results which have an application to the live animal.

The comparative balance method of determining true digestibility of phosphorus yielded values which were markedly different from those as determined by the radioisotope dilution technique.

LITERATURE CITED

LITERATURE CITED

Ammerman, C. B., A. L. Newmann, R. M. Forbes and H. W. Norton. 1954. Utilization of phosphorus from various inorganic sources by steers. J. Animal Sci. 13:974 (abstract).

Ammerman, C. B., H. W. Norton, H. M. Scott and A. H. Nesbit. 1960. Rapid assay of inorganic phosphates for chicks. Poultry Sci. 39:245-250.

Ammerman, C. B., R. M. Forbes and U. S. Garrigus. 1955. Utilization of phosphorus from various inorganic sources by lambs. J. Animal Sci. 14:1192 (abstract).

Ammerman, C. B., R. M. Forbes, U. S. Garrigus, A. L. Newmann, H. V. Norton and E. E. Hatfield. 1957. Ruminant utilization of inorganic phosphates. J. Animal Sci. 16:796.

Anderson, Russell, Edmund Cheng and Wise Burrough. 1956. A laboratory technique for measuring phosphorus availability of feed supplements fed to ruminants. J. Animal Sci. 15:489.

Barrentine, B. F., L. A. Maynard and J. K. Loosli. 1944. The availability of calcium and phosphorus of defluorinated rock phosphate for the rat. J. Nutr. 27:35.

Baxter, Harold. 1957. The effect of ortho, meta, pyro and phytin phosphates upon cellulose digestion by rumen microorganisms in vitro. M. S. Thesis, University of Tennessee.

Burroughs, W., A. Latona, P. Depaul, P. Gerlaugh and R. M. Bethke. 1951. Mineral influences upon urea utilization and cellulose digestion by rumen microorganisms using the artificial rumen technique. J. Animal Sci. 10:693.

Burroughs, W., A. Raun, E. Cheng and C. C. Culbertson. 1956. Different kinds and amounts of phosphorus in fattening cattle rations. Iowa State College A. H. Leaflet 209:1.

Chapman, H. L., J. Kastelic, B. C. Ashton and D. V. Catron. 1955. A comparison of phosphorus from different sources for growing and finishing swine. J. Animal Sci. 14:1073.

Cheng, E. W., Glen Hall and Wise Burroughs. 1955. A method for the study of cellulose digestion by washed suspension of rumen microorganisms. J. Dairy Sci. 38:1225.

Comar, C. L., R. A. Monroe, W. J. Visek and S. L. Hansard. 1953. Comparison of two methods for determining endogenous fecal calcium. J. Nutr. 50:459.

Comar, C. L., S. L. Hansard, S. L. Hood, M. P. Plumlee and B. F. Barrentine. 1951. Use of calcium-45 in biological studies. Nucleonics 8:19.

Crampton, E. W. and L. A. Maynard. 1938. The relation of cellulose and lignin content to the nutritive value of animal feeds. J. Nutr. 15:383.

Creech, B. G., B. Reid and J. R. Couch. 1956. Evaluation of dicalcium phosphate supplement as a source of phosphorus for chicks. 1. Comparison of dicalcium and tricalcium phosphates as a source of phosphorus in chick and poult rations. Poultry Sci. 35:654-658.

Dew, M. L., G. E. Stoddard and G. O. Bateman. 1954. Phosphorus supplements made more palatable with salt. Utah Agr. Exp. Farm and Home Sci. 15:36.

Duckworth, J. and R. Hill. 1953. The storage of elements in the skeleton. Nutr. Abst. Revs., 28:1.

Ellis, N. R. and C. A. Cabell. 1945. Biological availability of defluorinated phosphates. Feedstuffs. January 20.

Ellis, N. R., C. A. Cabell, W. P. Elmslie, G. S. Grops, P. H. Phillips and Dorthorey E. Williams. 1945. Nutritive evaluation of defluorinated phosphates and other phosphorus supplements. III. Utilization experiments with rats. J. Assoc. Office Agr. Chem. 28:129.

Fiske, C. H. and Y. Subbarrow. 1925. The colorimetric determination of phosphorus. J. Biol. Chem. 66:365.

Gaddy, C. D. 1960. Influence of phosphorus supplements upon cellulose digestion by rumen microorganisms and ration digestibility by sheep. M. S. Thesis, University of Tennessee.

Gardiner, E. E., H. E. Parker and C. W. Carrick. 1959. Soft phosphate in chick rations. Poultry Sci. 38:721-727.

Gillis, M. B., K. W. Keans and R. A. Collins. 1957. Comparative metabolism of phytate and inorganic P-32 by chicks and poults. J. Nutr. 62:13.

Gillis, M. B., L. C. Norris and G. F. Heuser. 1951. Biological availability of inorganic phosphates. Poultry Sci. 30:914.

Gillis, M. B., L. C. Norris and G. F. Heuser. 1954. Study on the biological value of inorganic phosphates. J. Nutr. 52:115.

Gillis, M. B., L. C. Norris and G. F. Heuser. 1948. The utilization by the chick of phosphorus from different sources. J. Nutr. 35:193-207.

Gillis, M. B., L. C. Norris and G. F. Heuser. 1953. Phosphorus metabolism and requirements of hens. Poultry Sci. 32:977.

Glass, B. G. 1952. Mineral Metabolism. John Hopkins Press, Baltimore.

Grau, C. R. and P. A. Zweigart. 1953. Phosphate clay as a phosphorus source for chicks. Poultry Sci. 32:500-503.

Greenberg, E. M. 1939. Mineral metabolism, calcium, magnesium, and phosphorus. Ann. Rev. Biochem. 8:269.

Gullickson, T. W. and F. C. Olson. 1946. Defluorinated rock phosphates as a phosphorus supplement to the ration of dairy cattle. J. Dairy Sci. 29:317.

Hall, O. G. 1955. Unidentified factors stimulatory to cellulose digestion by rumen microorganisms. PhD. Thesis, Iowa State University, Ames, Iowa.

Hall, O. G., C. D. Gaddy and C. S. Hobbs. 1961. Influence of phosphorus supplements on cellulose digestion by rumen microorganisms and on ration digestibility by sheep. J. Animal Sci. 20:395 (abstract).

Hall, O. G., H. D. Baxter and C. S. Hobbs. 1961. Effects of phosphorus indifferent chemical forms on in vitro cellulose digestion by rumen microorganisms. J. Animal Sci. 20:817.

Hansard, S. L. 1952. Metabolism procedures and their significance in radioisotope studies with farm animals. AEC-TID, 5098.

Hansard, S. L. 1951. Radioisotope procedures with farm animals. Nucleonics 9:13.

Hansard, S. L., C. L. Comar and M. P. Plumlee. 1951. Radioisotope procedures with farm animals. II Quantitative administration and blood sampling techniques. Nucleonics 9:38.

Hansard, S. L., W. O. Butler, C. L. Comar and C. S. Hobbs. 1953. Blood volume in farm animals. J. Animal Sci. 12:402.

Hansard, S. L. 1956. Tracer methods in mineral nutrition research. AEC-TID 7512:31.

Hodgson, C. W., R. F. Johnson, A. C. Wiese and C. W. Hickman. 1948. A comparison of steamed bone meal and defluorinated superphosphate as phosphorus supplements for fattening steers. J. Animal Sci. 7:273.

Jordan, E. E., M. H. Kennington, M. P. Plumlee and W. M. Beeson. 1956. Phosphorus supplements for swine. Purdue University Agr. Exp. Sta., Mimeo., A. H. 215.

Jordan, W. H., E. B. Hart and A. J. Patton. 1906. A study of the metabolism and physiological effects of certain phosphorus compounds with milk cows. Am. J. Physiol. 16:268.

Kleiber, Max, A. H. Smith, N. P. Ralston and A. L. Black. 1951. Radiophosphorus as tracer for measuring endogenous phosphorus in cows' feces. J. Nutr. 45:253.

LeGrande, C. E. and A. D. Tillman. 1961. Utilization of phytin phosphorus in wheat bran by sheep. J. Animal Sci. 20:1961.

Lofgreen, G. P. 1960. The availability of the phosphorus in dicalcium phosphate, bone meal, soft phosphate and calcium phytate for mature wethers. J. Nutr. 70:58.

Lofgreen, G. P. and M. Kleiber. 1953. The availability of phosphorus in alfalfa hay. J. Animal Sci. 12:366.

Long, T. A., A. D. Tillman, H. B. Nelson, W. D. Gallup and B. Davis. 1956. Availability of phosphorus in mineral supplements for beef cattle. J. Animal Sci. 15:1258.

Lyke, W. A. 1959. Preparation and analysis procedures for radioactive and stable calcium and phosphorus and for separation of radioactive calcium, phosphorus and strontium. UT-AEC Laboratory, Oak Ridge (unpublished mimeo).

Mathur, M. L. 1953. Assimilation of phytin phosphorus by dairy cows. Indian J. Vet. Sci. 23:243-248.

Miller, M. W. and V. V. Joukovsky. 1953. Availability of phosphorus from various phosphate materials for chicks. Poultry Sci. 32:78-81.

Raun, A., E. Cheng and W. Burroughs. 1956. Phytate phosphorus hydrolysis and availability to rumen microorganisms. J. Agr. Food Chem. 4:869.

Reed, O. E. and C. F. Huffman. 1930. The results of five year mineral feeding with dairy cattle. Mich. Agr. Exp. Sta. Tech. Bul. 105:3-63.

Reid, R. L., M. C. Franklin and E. G. Hallsworth. 1947. The utilization of phytate phosphorus by sheep. Australian Vet. J. 23:136.

Reynolds, D. S., S. L. Hill and K. D. Jacob. 1944. Dilute hydrochloric acid as a solvent for phosphates with special reference to defluorinated phosphates and other materials used as phosphorus supplements for livestock. J. Assoc. Office Agr. Chem. 27:559.

Satchidanandam, V. 1961. Utilization of phosphorus from dicalcium phosphate and defluorinated phosphate supplements by rumen bacteria in vitro and by lambs fed a semi-purified ration. M. S. Thesis. University of Tennessee.

Schroder, J. D. 1957. Some physiological effects of estrogens upon ruminants. PhD. Thesis. Oklahoma Agr. and Mech. College.

Smith, S. E. and M. B. Wise. 1957. How much phosphorus for dairy calves? N. Y. State Agr. Exp. Farm Res. 23:13.

Snedecor, G. W. 1956. Statistical methods. The Iowa State College Press, Ames, Iowa.

Stoddard, G. E. and H. Mickelsen. 1961. Phosphorus intake depends on taste. Utah Farm and Home Sci. 22:103.

Theiler, A., H. Green and P. J. duToit. Dicalcium phosphate and phosphoric acid as phosphorus source for beef cattle. J. Animal Sci. 17:100.

Thompson, Alan. 1957. The influence of aluminum and zinc upon the absorption and retention of calcium and phosphorus in lambs. M. S. Thesis. University of Tennessee.

Tillman, A. D. and J. R. Brethour. 1958a. Utilization of phytin phosphorus by sheep. J. Animal Sci. 17:104.

Tillman, A. D. and J. R. Brethour. 1958b. Ruminant utilization of sodium meta ortho and pyrophosphates. J. Animal Sci. 17:792.

Tillman, A. D. and J. R. Brethour. 1958c. Dicalcium phosphate and phosphoric and as phosphorus sources for beef cattle. J. Animal Sci. 17:100.

Tillman, A. D., A. Long, A. B. Nelson, W. D. Gallup and Bill Davis. 1956. Phosphorus supplements for cattle and sheep. Misc. Publ. No. MP-45 Okla. A & M College, pp. 73-79.

Toit, P. J., A. I. Malan and H. H. Green. 1930. Studies in mineral metabolism; phosphorus in the sheep industry, preliminary report. S. Africa Agr. Dept. Rpt. 2:313.

Visek, W. J., R. A. More, E. W. Swanson and C. L. Comar. 1953. Determination of endogenous fecal calcium in cattle by a simple isotope dilution method. J. Nutr. 50:23.

Wilcox, R. A., C. W. Carlson, W. Kohlmeyer and G. F. Gostler. 1954. The availability of phosphorus from different sources for poults fed purified diets. Poultry Sci. 33:1010.

Wise, M. B., R. A. Wentworth and S. E. Smith. 1961. Availability of the phosphorus in various sources for calves. J. Animal Sci. 20:329.